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Kinetics of [2–^{14}C]Glycine Decomposition and Its Incorporation into Humus Fractions of Sierozem

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A model is presented for the decomposition of [2–^{14}C]glycine in soil and for the accompanying labeling of microbial biomass; the model accounts for more than 98 percent of the dispersion of values of solving $^{14}CO_2$. The half-lives of [2–^{14}C]glycine and of the microbial biomass in sierozem were 0.61 and 8.8 days, respectively. Addition of 1 percent salts reduced only the initial rate of glycine decomposition. At 10 percent salinization the total amount of $^{14}CO_2$ evolved over two months decreased and the kinetics of evolution changed.

Key words: Sierozem, humus fractions, glycine decomposition, model for decomposition

The behavior of low-molecular-weight organic compounds in soils is receiving increased attention. A major number of works are devoted to the transformation of amino acids, which are crucial in conversion of nitrogen-containing organic residues in soil and in other biochemical processes [4, 35, 39]. The rate of decomposition of amino acids characterizes organic nitrogen mineralization and the level of biological activity of the soil; the incorporation of amino acids in humus reflects the rate and completeness of humus restoration. Transformation of amino acids in sierozem is practically unstudied. Humus formation in sierozem, though considerably widespread [25], is as yet inadequately explored in comparison to that in podzolic and chernozemic soils.

Improvident irrigation of sierozems often leads to resalinization [9, 13]. Abundance of salts significantly reduces the biological activity of soil and affects the content of the soil microflora [18, 27, 32, 33]. There is evidence that plants [23] and microorganisms [26] resist the increased osmotic pressure of soil solution at the expense of increasing the intercellular concentration of organic acids, mainly amino acids. Therefore the behavior of amino acids in soil under various conditions of salinization is of prime interest. The effect of chemical transformations and of the extent of soil salinization on the processes of mineralization and humification is also practically unstudied.

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The goals of the present work were

- To study the dynamics of [2–^{14}C]glycine decomposition in sierozem and to assign the process of $^{14}CO_2$ evolution into specific stages
- To develop a model for decomposition of amino acids in soil to characterize each of the proposed stages and to describe adequately the temporal behavior of each component
- To assess the influence of salts ($NaHCO_3$, $CaSO_4 \cdot 2H_2O$, NaCl, and Na_2SO_4) on the dynamics and amount of $^{14}CO_2$ evolution from the soil and on the parameters of the model
- To assess the incorporation of ^{14}C from the decomposition of [2–^{14}C]glycine into humus fractions of sierozem under various conditions of salinization, both in the presence of microorganisms and after complete sterilization of the soil

Materials and Methods

To solve the problems posed in the laboratory, experiments were performed on the interaction between [2–^{14}C]glycine and soil. Soil samples from the upper layer (0–20 cm) of virgin light sierozem [14] from the Lomakinskiy plateau area of the Dzhizak steppe (Uzbekistan) were taken for the experiment. This soil has the following features: organic carbon content, 0.55 percent; total nitrogen content, 0.09 percent; $CaSO_4 \cdot 2H_2O$ content, 0.05 percent; $CaCO_3$ content, 7.32 percent; pH (1:2.5) = 8.45. The ion contents in water extract (1:5), in meq/100 g of soil, are: Cl^-, 0.01; SO_4^{2-}, 0.12; HCO_3^-, 0.60; Ca^{2+}, 0.45; Na^+, 0.03.

The interaction of [2–^{14}C]glycine with nonsalined soil and with soil salined with the salts $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, NaCl, and Na_2SO_4 at concentrations 0.1, 1, and 10 percent of the soil weight was studied with and without sterilization.

The experiments were performed by the following procedure. Twenty-gram portions of soil sifted through a screen with 2-mm diameter holes were placed into 50–ml conical flasks together with the listed salts in the amounts calculated for each variant. Water was added into each flask up to 60 percent of the total moisture content of soil (with total moisture content being 45 percent of the soil weight), and the flasks were set aside for a week for the microorganisms to develop. The soil was sterilized by three cycles of autoclaving at 1 atm for 1 hour, then 0.2 ml of phenol solution was added. The experiments were performed in duplicate.

A plastic cup with 1 ml of 1 N NaOH was placed into each soil-containing flask to capture CO_2. Then 0.5 ml of glycine solution containing 750 μg (10 μmoles) of glycine with a total radioactivity of 413 kBq was applied into the soil in each flask. As soon as the glycine was introduced, the flasks were closed tightly with rubber plugs. The amount of introduced glycine was roughly equal to the amino acid content in the soils [8, 12, 15], so the additional slight source of energy could not change significantly the settled proportion of groups of microorganisms and the number of these groups. The microbiological activity of the soil was evaluated by radio respirometry.

Throughout the experiments, the alkali adsorbing evolved $^{14}CO_2$ was exchanged in the cups through special holes in the plugs, and the radioactivity of the alkali samples were measured.

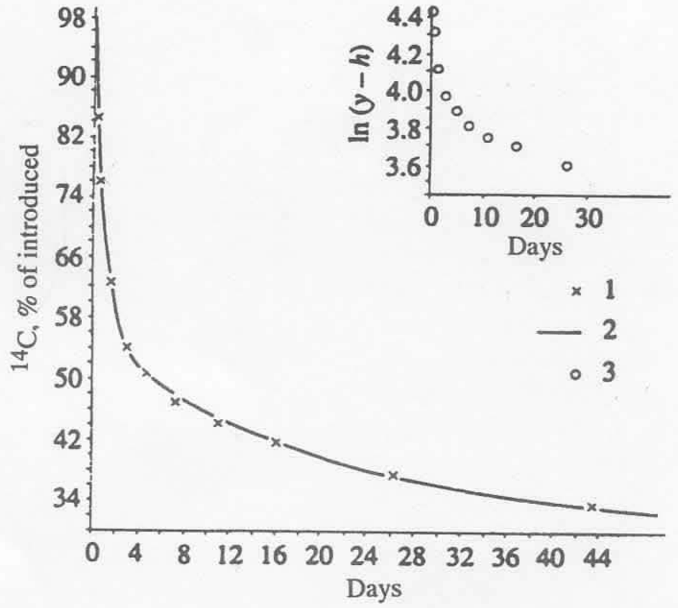


Fig. 1. Experimental values of ^{14}C content in soil (1), theoretical curve calculated from them (2), and logarithmic linearization of the experimental values (3).

An experiment was held to be complete when $^{14}CO_2$ evolution in all the variants was not greater than 0.2 percent of the introduced radioactivity per day (this occurred after 55 days).

After incubation was complete, consecutive exhaustive extractions were performed with 1 N KCl (1.5), 0.1 N NaOH (1:10), and 0.1 N $Na_4P_2O_7$ + 0.1 N NaOH (1:10) [30]. Free amino acids were assumed to be transferred into the KCl extract; humic acids complexed with alkaline metals and those loosely bound with sesquioxides were assumed to be transferred into the NaOH extract; Humic acids complexed with Ca^{2+} and sesquioxides were assumed to be transferred into the pyrophosphate extract. Humic acids of NaOH extract were recovered by evaporation, and nonspecific low-molecular-weight compounds were separated using Sephadex G-10 (separation limits 0–700) [16]. The traditional methods of humus isolation and fractionation were abandoned for the following reasons. First, it was necessary to extract as completely as possible the initial [2–^{14}C]glycine, which had very high specific activity in comparison with the other fractions of organic matter; if mixed with the other fractions even in small amounts, the distribution of the label would be severely affected. Second, the traditional method of separation into humic and fulvic acids by taking the pH to 1 [16] may cause a hydrolysis of weak neogenic bonds.

In all the extracts and in the residual soil, the radioactivity of ^{14}C was determined using Bray and ZhS-13n scintillators and the RackBeta model 1219 (LKB) beta spectrometer. Also, total carbon was determined by the Tyurin method using photometry [11].

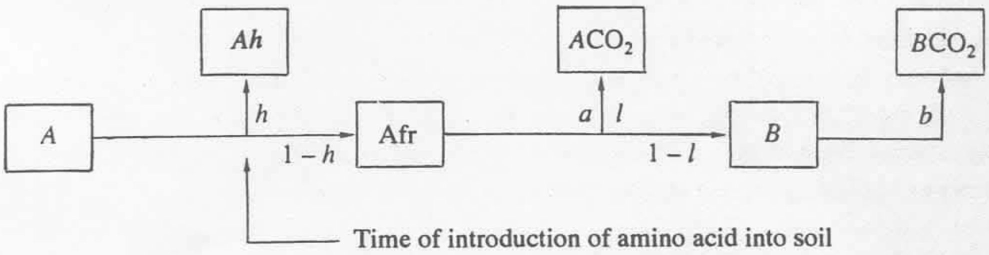
Results and Discussion

A model for [2–^{14}C]glycine decomposition in soil. After [2–^{14}C]glycine was introduced into soil, a very fast evolution of $^{14}CO_2$ was observed. In Fig. 1, the ^{14}C content of the soil during the experiment is shown, calculated from the difference between the introduced amount (100 percent) and the total amount of evolved $^{14}CO_2$. After 2–3 days, the rate of evolution decreased drastically and did not exceed 0.3 percent per hour. Thus the process of $^{14}CO_2$ evolution can be assigned to at least two stages which are sharply distinguished by the rates. The first stage consists of the fast decomposition of glycine itself, and it determines the overall rate of mineralization during 1–2 days after application of the amino acid into the soil. This stage can be characterized by the average rate of glycine utilization by microorganisms, which may range from 0.5 to 2 percent of the introduced amount per hour. The second stage consists of slow decomposition, and it becomes clearly apparent 2–3 days after introduction of the glycine into the soil (the rate may range from 0.01 to 0.2 percent evolution of $^{14}CO_2$ per hour). The appropriateness of assigning the processes of decomposition of plant and microbial residues into two stages, that is, a fast and a slow stage, is supported by a number of investigations [4, 31, 39], although the stages may differ significantly for different compounds.

In the sterile soil variants, after [2–^{14}C]glycine was introduced into the soil, a part of the amino acid content (33–35 percent of the introduced amount) became chemically linked with humus and could not be extracted by 1 N KCl. This part of the label was practically not utilized by microorganisms during 2 months of the experiment. Similar evidence for the sorption of amino acids by soils [21, 24, 35] and of the fact that the rate of decomposition is much lower for adsorbed compounds [21, 29, 39] have also been obtained in some other investigations (according to [24], glycine was the only amino acid not adsorbed by clay minerals, for example, by montmorillonite and kaolinite). Moreover, it was determined in the sterile variants that there was no purely chemical oxidation of the second carbon atom of glycine to CO_2.

Thus the transformation of the amino acid after its introduction into soil can be represented as follows. A part of amino acid is adsorbed by humus in a very short time. The remaining amino acid can be utilized by microorganisms [20], and thus to be involved in metabolism accompanied by $^{14}CO_2$ evolution. After the microorganisms die off, ^{14}C included into them is also oxidized to $^{14}CO_2$.

The scheme for the decomposition of the amino acid in soil consists of one chemical stage and two biological stages



where A is the initial amount of the amino acid (100 percent), Ah is the amount of the amino acid adsorbed by humus right after its introduction into the soil, Afr is the amount of free amino acid, h

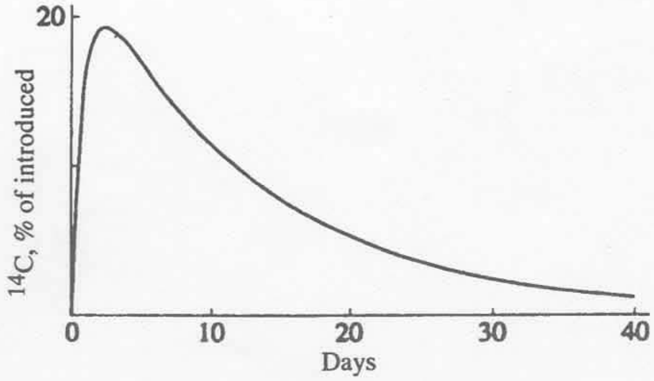


Fig. 2. Curve for ^{14}C content in microbial biomass calculated by the model.

is the fraction of the amino acid firmly adsorbed by humus, *a* is a constant of the rate of amino-acid utilization by microorganisms, *l* is the fraction of the free amino acid utilized by microorganisms for anabolism, *B* is the produced labeled biomass of mciroorganisms, *b* is a constant of the rate of decomposition of the microorganisms, ACO_2 is labeled carbon dioxide evolved in utilizing of free amino acid by microorganisms, BCO_2 is labeled carbon dioxide evolved in the decomposition of dead microorganisms *B*.

The first stage, the interaction between the amino acid and humus, is fast in comparison to the other two biological stages. According to the literature and to the results of our previous investigations, this stage lasts no longer than 30–60 minutes after the addition of the amino acid to the soil [4, 21, 24, 35]. Thus we may assume that separation of the amino acid into free and soil-linked forms should be completed by the beginning of glycine utilization by the microorganisms.

If the next two stages, which consist of $^{14}CO_2$ evolution as such, proceed in accordance with the scheme outlined above, and if decomposition of the individual compounds obey first-order kinetics [5, 17, 38, 39], then the dynamics of ^{14}C content in each of the matters is described by the following equations [17]:

$$\frac{dACB}{dt} = aACB(t) \tag{1}$$

$$\frac{dB}{dt} = laA\,CB(t) - bB(t) \tag{2}$$

$$\frac{dACO_2}{dt} = (1 - l)\,aACB(t) \tag{3}$$

$$\frac{dBCO_2}{dt} = bBCO_2(t) \tag{4}$$

where *t* is for time.

Table 1

Constants of Decomposition (day^{-1}) and Half-Lives (days) of [2–^{14}C]Glycine and of the Produced Labeled Microbial Biomass in Relation to Conditions of Salinization (for $h = 0.3$ and $l = 0.36$)

Variant	a			b			$T_{1/2}$ (a)			$T_{1/2}$ (b)		
Control	1.14			0.079			0.61			8.8		
	Salt concentration, %											
	0.1	1	10	0.1	1	10	0.1	1	10	0.1	1	10
NaCl	1.1	0.84	0.1	0.05	0.04	0.03	0.6	0.8	47	14	26	22
$NaHCO_3$	1.7	0.51	0.05	0.06	0.08	–	0.47	1.4	13	12	13	–
Na_2SO_4	2.2	1.4	0.22	0.05	0.08	0.02	0.46	0.51	3.2	14	8.8	40
$CaSO_4 \cdot 2H_2O$	0.9	1.2	1.2	0.04	0.06	0.04	0.77	0.56	0.6	20	11	17

Note. A dash means that the model gives negative values for the rates of decomposition because of a prolonged lag phase.

These differential equations have analytical solutions, thus allowing algebraic equations to be written giving the content of each labeled product of the transformation of the initial amino acid at particular times [5].

Therefore the total amount of carbon dioxide evolved from soil is described by the equation

$$^{14}CO_2(t) = ACO_2(t) + BCO_2(t) \tag{5}$$

and after solving the differential equations and some transforms we obtain

$$^{14}CO_2(t) = 100(1 - h)\,(1 - \exp(-at))(1 - l\exp(-bt)) \tag{6}$$

The label content in soil is described by the equation

$$^{14}C_{\text{soil}}\,(t) = 100 - {}^{14}CO_2(t) \tag{7}$$

and, respectively,

$$^{14}C_{\text{soil}}\,(t) = 100(1 - (1 - h)\ \times\ (1 - \exp(-at))\,(1 - l\exp(-bt))) \tag{8}$$

The constant h can be found experimentally from the amount of the label not extracted by 1 N KCl (exhaustive extraction) from sterile soil. In our experiment, the fraction of the ^{14}C-amino acid in humus was 34 percent.

It is rather difficult to evaluate the coefficient l by the results of a simple experiment, for there is no acceptable method for complete isolation of microorganisms from the mineral soil

Table 2

Percentage Distribution ^{14}C among Fractions of Organic Matter of Sierozem after 55 Days Incubation of [2–^{14}C]Glycine in Unsterilized Soil (numerator) and in Sterilized Soil (denominator)

Variant	Control	Salt concentration, %											
		NaCl			$NaHCO_3$			Na_2SO_4			$CaSO_4 \cdot 2H_2O$		
		0.1	1	10	0.1	1	10	0.1	1	10	0.1	1	10
$^{14}CO_2$	70/2.0	67/1.3	65/2.4	35/1.1	68/1.6	68/3.1	27/0.5	66/2.7	69/3.3	63/3.0	64/2.5	68/2.2	66/1.9
KCl	0.7/66	0.7/65	1.3/66	14/65	1.4/68	7.3/69	48/83	0.6/63	0.6/64	4.0/67	0.9/65	0.8/66	0.7/64
NaOH	14/18	15/18	14/19	30/26	12/16	9.2/15	6.8/6.0	15/18	15/18	13/15	16/18	14/18	7.6/15
$Na_4P_2O_7$ + + NaOH	2.7/6.0	3.2/4.5	3.5/4.7	4.4/1.9	2.6/4.4	2.8/3.0	1.8/1.2	3.1/5.7	2.2/5.4	3.8/4.5	2.3/5.2	2.4/5.1	11/14
Humin	10/3.3	12/3.8	12/3.5	6.1/3.4	12/3.9	8.4/3.7	7.1/1.5	9.3/3.5	7.9/4.2	8.9/4.1	9.5/3.6	13/3.4	7.2/3.8
Losses	2.6/4.7	2.1/7.4	4.2/4.4	10.5/2.6	4.0/6.1	4.3/6.2	9.3/7.8	6.0/7.1	5.3/5.1	7.3/6.4	7.3/5.7	1.8/5.3	7.5/1.3

matrix and from low-molecular-weight organic compounds simultaneously. Therefore information from the literature on this problem was used.

It is conventional in the literature on biotechnology to call this coefficient an economical coefficient [7]. For processes running under optimal conditions in biotechnological industry, it may range from 40 to 90 percent [3, 6, 7]. But it should be taken into account that an economical coefficient is a ratio of the product weight to the weight of substrate, and it is not a ratio between the quantities of carbon atoms in the initial substrate and in the synthesized products. This coefficient is overestimated by 20–40 percent, because the weight of the product increases at the expense of higher extent of its oxidation as compared to the substrate. Thus, for strongly oxidized substrates such as sugars (glucose, sucrose), this coefficient is 40–50 percent, and for the less oxidized products (ethanol, paraffins), it increases to 65–90 percent [6, 7].

The energy exchange in microscopic fungi is most perfect among microorganisms, and their economical coefficient of substrate utilization under natural conditions is 50–60 percent (Waksman, 1944; Foster, 1950, cited from [1]). Bacteria play a crucial role in substrate utilization in sierozem; their economical coefficient is lower and under optimal conditions it is 35 percent [19]. For soil microorganisms, this coefficient should be still lower because of the permanent shortage of easily accessible substrate.

The C/N ratio of 20–25 is optimal for the development of microorganisms in plant residues (optimal in the sense that nitrogen losses are minimal) [2], and for microbial cells the optimal C/N ratio is approximately 5 [1]. Taking this into account, we can assume that the coefficient of carbon utilization for anabolism is approximately 20–25 percent. In this model describing decomposition of glucose and microbial cells in soil, this coefficient is taken to be 60 percent for glucose and 30–40 percent for easily decomposed compounds [38, 39], which seems to be an overestimation in our opinion. Odum [10] believes that the coefficient of energy transmission via the food chain amounts to only 10 percent (the energy-transmission coefficient is lower than the carbon-transmission coefficient because total oxidation of compounds increases in the process of passing through the food chain). There is reason to believe that microorganisms utilize more intensively in anabolism the second carbon atom (bound with nitrogen) of amino acids than the carbon of the amino acid carboxyl, which is more oxidized [4, 20].

In view of the considerable variety in the literature on this problem, we determined the economical coefficient from the experimental data by solving Eqs. (6) and (8) for this coefficient using nonlinear regression. For different variants of the experiment, it ranged from 32 to 40 percent. In further calculations, we assume that the coefficient of utilization of the second carbon atom of glycine by microorganisms for anabolism is constant for all the experimental variants and that its value is 36 percent, in agreement with the results of Babiuk et al. [19] obtained experimentally.

The ^{14}C content from the left side of Eq. (8) is calculated from the difference between 100 percent and the amount of evolved $^{14}CO_2$. The calculations were performed using cumulative graphs of $^{14}CO_2$ evolution instead of daily ones, thus permitting smoothing of some irregularities in the evolution of the labeled carbon dioxide and reduction in the error in the calculation of the coefficients. Equations (6) and (8) give the same coefficients for the rates of decomposition, but in solving Eq. (8), unlike Eq. (6), one can use the fact that the error is proportional to the activity.

From Eq. (8), the characteristic constants for the rates of decomposition of the initial amino acid and of biomass were calculated using nonlinear regression and the least-squares method with allowance for the effect of error of each measurement (the error is proportional to the value). The resulting coefficients were 1.14 and 0.079 day^{-1}; they correspond to the half-lives 0.61 and 8.8 days. The half-life of glycine indicates that the rate of decomposition of the free amino acid in soil is very high. Similar half-lives were obtained by a number of researchers for low-molecular-weight organic compounds [22] and for the biomass of dead microorganisms [29, 36, 39], although there is also evidence for a longer half-life for dead labeled microbial biomass [31, 34].

From the rate of biomass decomposition, we obtained the lifetime of the microorganisms of 15–20 days, and this is somewhat longer than that implied by the literature indicating about 30–40 generations of microorganisms in soil per vegetative period [2]. These data were obtained by computation on the basis of energetics. It is worth noting that, despite the fact that there is sufficient information in the literature about the quantity and the species composition of microorganisms in soil, there are almost no data about mean lifetime of microorganisms in soil.

The proposed model for the decomposition of low-molecular-weight organic compounds in soil has a number of merits in comparison with the exponential model often used for description of decomposition processes in soil.

The proposed model for the decomposition of low-molecular-weight organic compounds in soil has a number of merits in comparison with the exponential model often used for description of decomposition processes in soil. For example, it is proven by a great number of investigations that the decomposition process in soil is not governed by a simple exponential law because the coefficient of decomposition is not constant, and for different stages these coefficients may differ by more than two orders of magnitude [4, 20, 28, 31, 37, 39]. Taking the logarithms of the experimental values of ^{14}C content in soil does not make the curve linear (Fig. 1). The half-lives found by the proposed model are not weighted averages of those for several processes, as they are for calculations on the basis of the simple exponential law. They characterize more precisely the rate of decomposition of both the initial compound and its first nongaseous metabolite.

Here are some statistical features of the model: the efficiency index r^2 (the ratio of deviation derived from the equation to the total deviation) was no less than 98 percent in all the experimental variants, and the Student test index was no less than 3000 (for the simple exponential equation these indices are 40 and 50 percent, respectively). The standard deviations of a and b were less than 15 percent, and the constants of the decomposition rates in different repetitions varied within the range of 30 percent.

Using this system of equations and the calculated coefficients for decomposition of initial amino acid and biomass, one can determine the dynamics of ^{14}C content in each block of the model, in particular, in the biomass. Figure 2 shows such theoretical curve with a typical peak of ^{14}C content in the composition of biomass within a certain period after the addition of the amino acid into the soil. A similar peak for label inclusion into biomass or into humus fractions for various periods was observed in a number of other experiments [4, 16, 29, 31, 34, 37, 39]. The position and amplitude of the peak are completely defined by the ratio between the decomposition rate constants for the initial amino acid and biomass and by the portion of amino acid utilized by the microorganisms for anabolism [5]. Judging from the calculated decomposition constants a and b and from the assumed value of l, the maximum ^{14}C inclusion into biomass should be attained 2.5 days after the beginning of [2–^{14}C]glycine decomposition, and it should comprise 19.5 percent of the introduced radioactivity [5]. In some other works, a similar maximum was observed after 2–4 days [29, 34], and it ranged from 4 [31, 37] to 30 percent [29]. By virtue of the long lapse of time between the onset of the experiment and the first sampling, the maximum value of the label inclusion into biomass might be missed in some works [31, 37]. This may explain why only 4 percent of the radioactivity was found in the composition of microorganisms.

The proposed model is rather simple. It is characterized by only four parameters, each having clear physical meaning.

The model also has some shortcomings and restrictions:

- The coefficient b represents an integral constant for the rate of microorganisms biomass restoration not only for the first generation, but also for consequent generations (whereas the contribution of each subsequent generation of microorganisms does not exceed l^n, where n is the generation number. For the second generation of microorganisms with the assumed value of l, this contribution does not exceed 13 percent, and for the third, it does not exceed 5 percent). Therefore, the half-lives obtained for microbial biomass are somewhat overestimated (by 15–20 percent).

- The proposed model cannot be employed when the investigated period exceeds considerably the time of almost complete decomposition of biomass, because in these periods $^{14}CO_2$ evolution is mainly from the amino acid already included into humus.

- The model does not take into account $^{14}CO_2$ evolved in the decomposition of amino acid included into humus (the total amount of $^{14}CO_2$ evolved from that pool during the experiment should not exceed 15–20 percent of the adsorbed amount, or 4–6 percent of the introduced radioactivity [18], which is not greater than experimental error).
- Conditions of decomposition (type of the soil, organic matter content, sorption properties of the soil, temperature, moisture content) are not accounted for in the model, although they should influence the constants of decomposition rates *a* and *b*. These factors can be subjected to further investigations.

Effects of salts on [2–^{14}C]glycine decomposition in soil. The presence of salts in soil affected the dynamics of evolution of the labeled carbon dioxide, the extent of the effects depending strongly on the type and amount of salt.

Addition of the salts at a concentration corresponding to 0.1 percent of the soil weight did not influence the rate or the amount of $^{14}CO_2$ evolved during the studied period (Fig. 3).

Salts added in amounts of 1 percent did not affect the total $^{14}CO_2$ evolution, although they slightly reduced the rate of evolution at the first stage (Fig. 3). This indicates fast adaptation of the microorganism complex in sierozem to the established conditions of salinization.

As in the control, in the variants with 0.1 and 1 percent added salts, the total amount of $^{14}CO_2$ evolved in the period of two months approached 70 percent of the introduced amount, and this is exactly the amount of ^{14}C extracted from soil by KCl in sterile variants. Thus extraction with KCl nearly completely removes the free amino acid that is easily accessible to the microorganisms. This fact can be used for determining the amount of easily accessible amino acids in soil and for development of techniques.

Addition of 10 percent NaCl or $NaHCO_3$ decreased the total amount of evolved $^{14}CO_2$ by a factor of 1.5–2 in comparison to the control (Fig. 3). Moreover, the kinetics of evolution changed considerably in the presence of 10 percent NaCl. This is due to the fact that chloride ions are toxic, and the microorganisms tolerant of salinization of this type develop slowly.

Introduction of $CaSO_4 \cdot 2H_2O$ at any studied concentration did not affect microbiological activity; this is probably due to the low solubility of the salt and, consequently, with the constancy of its concentration in soil.

The constants of rates of initial amino acid decomposition and of biomass restoration under various conditions of salinization were determined from the curves for ^{14}C content in soil (Table 1). In different variants of the experiment, the coefficients varied within the range of 30 percent.

Increasing the concentration of each studied salt (with the exception of $CaSO_4 \cdot 2H_2O$) increased the half-life of the labeled glycine in soil.

Effects of sterilization and salinization on ^{14}C incorporation into humus fractions. The distribution of ^{14}C among organic matter fractions after 55 days of incubation of [2–^{14}C]glycine

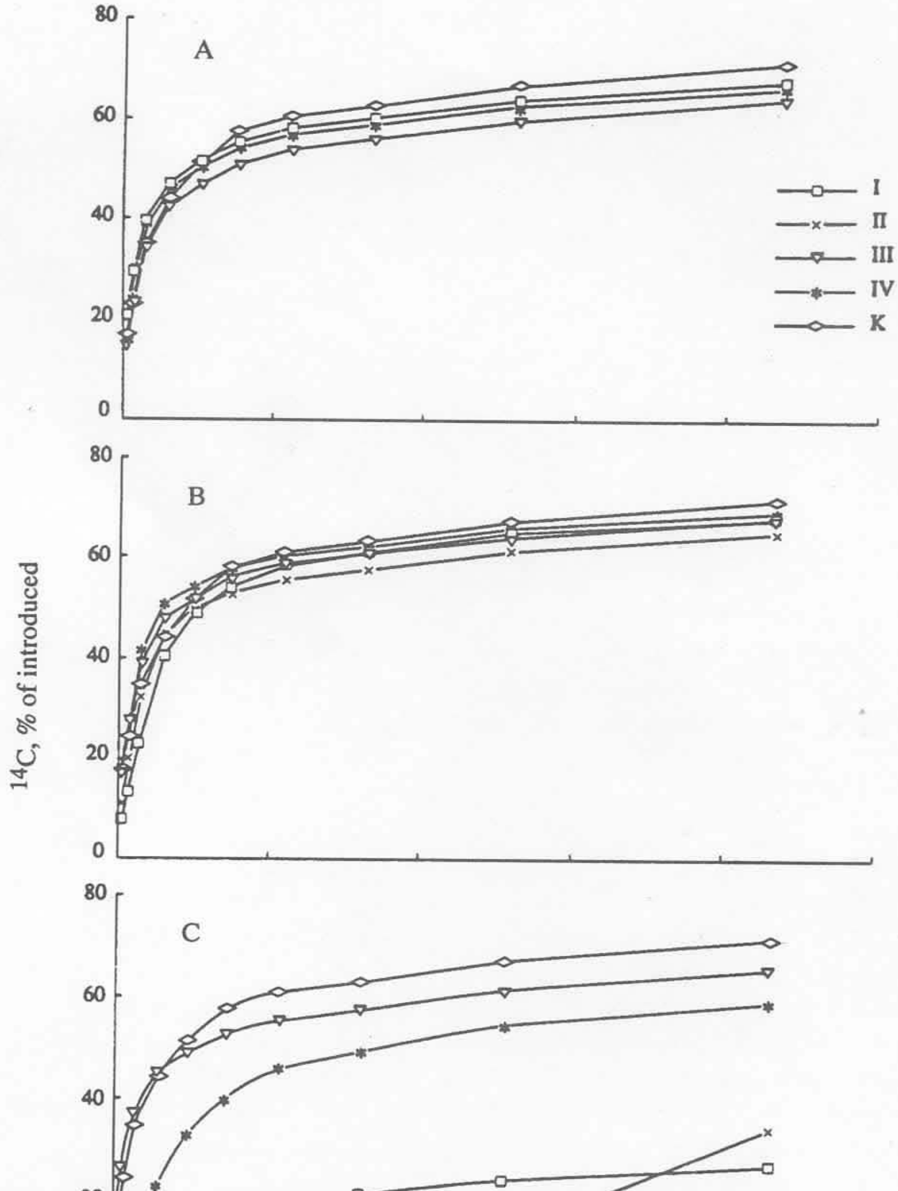


Fig. 3. Cumulative curves for $^{14}CO_2$ evolution during the decomposition of [2–^{14}C]glycine at 0.1 (A), 1 (B), and 10 percent (C) salinization of soil. Types of salinization: (I) $NaHCO_3$; (II) NaCl; (III) Na_2SO_4; (IV) $CaSO_4 \cdot 2H_2O$; (C, control) no salinization.

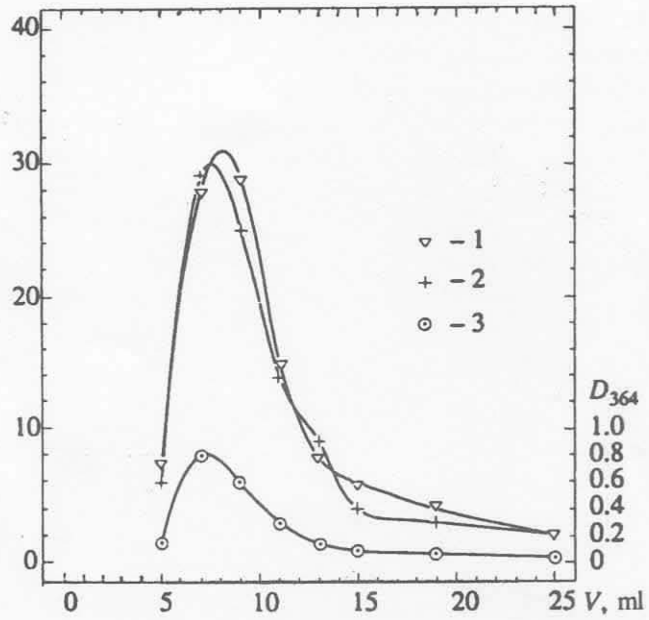


Fig. 4. Distribution curves for ^{14}C radioactivity ((1) and (2), sterilized and unsterilized variants, respectively) and for the optical density (D, 3) of humic acids from NaOH extract measured during the separation by molecular weight using Sephadex G-10. (Intermediate values are smoothed by a *Q*-spline.)

in soil is shown in Table 2. After incubation under sterile conditions, 66 percent of the ^{14}C could be extracted into KCl solution, and after normal development of microorganisms, this amount was reduced to 0.6 percent. This is evidence of the fact that amino acids extracted by 1 N KCl are easily accessible to the microorganisms, and they are utilized almost completely in 55 days of incubation. Composting of the amino acid in an unsterilized soil decreases carbon inclusion into all separable fractions (except for humin) as compared to that in sterile soil (Table 2). This resulted from oxidation of two-thirds of the initial ^{14}C in the amino acid and also from partial decomposition of the amino acid incorporated into humus.

For humin, which is the most inert fraction of soil humus, an increase in ^{14}C incorporation by a factor of 2–3 was observed as compared to sterile chemical interaction. Similar but lower inclusion of ^{14}C into humin was observed in variants with reduced microbiological activity (10 percent of NaCl and 10 percent of $NaHCO_3$). These results indicate that, after the amino acid is introduced into the soil, its primary chemical incorporation is into fractions which have a great number of active functional groups, such as fulvic and amino acids. Then, after microbiological transformation of neogenic peripheral fragments of molecules, the label redistributes among more inert fragments and fractions at the expense of splitting off from more labile ones. The increase of the label content in the nonextractable residue under composting is also attributable to inclusion of ^{14}C into the composition of cell walls of microorganisms, which are relatively inert, difficult to extract, and decompose more slowly than the microbial plasma.

No reliable evidence was found for the influence of the salts NaCl, $NaHCO_3$, and Na_2SO_4 at concentrations from 0.1 to 10 percent of the soil weight on ^{14}C incorporation into isolated fractions of soil organic matter under sterile conditions. Only 10 percent of $CaSO_4 \cdot 2H_2O$ or 10 percent of $NaHCO_3$ reliably ($P = 95$ percent) increased ^{14}C incorporation into fractions extracted by pyrophosphate and by KCl, due to increasing the share of these fractions themselves (of total carbon, according to Tyurin) [30].

As in the sterile variants, in the unsterilized variants, ^{14}C was included mainly into humus fractions with molecular weight greater than 500, that is, into specific humic acids. An effect of sterilization and salinization on the label distribution among high- and low-molecular-weight fractions of humic acids was not observed (Fig. 4).

Conclusion

(1) Decomposition of [2–^{14}C]glycine and of the labeled microbial biomass formed from it to $^{14}CO_2$ can be precisely described by a two-stage model. The calculated half-lives of the free amino-acid and of the biomass of microorganisms are 0.61 and 8.8 days, respectively.

(2) The proposed model for decomposition of low-molecular-weight matter in soil allows calculation of the incorporation of the label into the microorganism biomass. The peak of ^{14}C incorporation from the amino acid into the biomass occurs in 2.5 days, and it includes 19.5 percent of ^{14}C radioactivity introduced in the soil.

(3) The parameters of the proposed model for decomposition of low-molecular-weight organic matter in soil are sensitive to changes in the kinetics of decomposition caused by the addition of salts.

(4) Addition of salts in amounts of 1 percent of the soil weight lowered the initial rate of glycine decomposition, but it did not affect the total amount of evolved $^{14}CO_2$; 10 percent salinization (except that with $CaSO_4 \cdot 2H_2O$) decreased the total amount of $^{14}CO_2$ evolved during two months (salinization with NaCl and $NaHCO_3$— by two orders of magnitude).

(5) Glycine not linked with humus was utilized by microorganisms almost completely during the two-month experiment. Composition of amino acids in unsterilized soil decreased ^{14}C incorporation into fractions extractable by KCl, NaOH, and NaOH + $Na_4P_2O_7$ as compared to the sterile variants, but it increased significantly (by a factor of 2–4) the amount of ^{14}C included into nonextractable soil residue. This shows that a major part of the label from the low-molecular-weight compound is incorporated into the relatively more labile fractions of soil organic matter chemically, whereas only about one-third of the label is chemically incorporated into humin (which is more inert) and two-thirds of the label after microbiological transformation.

(6) The studied salts at concentrations up to 10 percent influence the incorporation of labeled carbon of the amino acid into humus fractions only by decreasing the microbiological activity of the soil.

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